UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

May 22, 2002

MEMORANDUM

SUBJECT: Lactofen - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Robert Fricke, Toxicologist
Reregistration Branch 2
Health Effects Division (7509C)

The Cancer Assessment Review Committee met on January 23, 2002 to evaluate the carcinogenic potential of Lactofen. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF LACTOFEN (SECOND REVIEW)
P.C. CODE: 128888

FINAL REPORT

21-May-2002
TXR # 0050184

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: _____
Robert F. Fricke

DOCUMENT PREPARATION: _____
Robert F. Fricke

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

List the Committee members

Karl Baetcke _____

William Burnam _____

Kerry Dearfield _____

Vicki Dellarco _____

Richard Hill _____

Nancy McCarroll _____

Esther Rinde _____

Joycelyn Stewart _____

Clark Swentzel _____

Linda Taylor _____

Duplicate Signature Page (FAX Copy)
contains Yin-Tak Woo's signature

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EXECUTIVE SUMMARY

On January 23, 2002, the Cancer Assessment Review Committee (CARC) met to reevaluate the cancer classification of lactofen in light of the conclusions [TXR No. 014590] of the Mechanism of Toxicity Assessment Review Committee (MTARC). The MTARC concluded that lactofen acts via a mechanism involving peroxisome proliferation as a result of activation of peroxisome proliferator-activated receptor α (PPAR α) activation.

On January 17, 2001, the MTARC reviewed the merits of the toxicological data supporting peroxisome proliferation as the proposed mode of action for lactofen. Based on the weight-of-evidence from guideline, as well as non-guideline mechanistic studies, the MTARC concluded that there are sufficient data to classify lactofen as a non-genotoxic hepatocarcinogen in rodents with peroxisome proliferation being a plausible mode of action.

This conclusion is based on the results of a special study in rodents which evaluated the biochemical and histopathological markers for peroxisome proliferation in livers (MRID No.: 45283904). In mice there was a dose-dependent increase in relative liver weights and liver enzyme activities used as biomarkers for peroxisome proliferation. Histological evaluations of the livers also revealed dose-dependent increases in nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining. These results not only show dose-dependent increases in the parameters measured, but more importantly, a non-linear dose-response curve.

The NOAEL for this study was established at 2 ppm (0.3 mg/kg/day), based on increased activities of liver enzymes and increased incidence of liver histopathological findings at the LOAEL of 10 ppm (1.5 mg/kg/day).

Based on the liver tumors seen in both sexes of the mouse, and in consideration of the mechanistic data provided, the CARC concluded that lactofen should be classified as **likely to be carcinogenic to humans at high enough doses to cause these biochemical and histopathological effects in livers of rodents but unlikely to be carcinogenic at doses below those causing these changes.** Further the margin of exposure (MOE) approach should be used for estimating human cancer risk, using a NOAEL of 2 ppm (0.3 mg/kg/day).

I. INTRODUCTION

On January 23, 2002, the Cancer Assessment Review Committee (CARC) met to evaluate the conclusions of the Mechanism of Toxicity Assessment Review Committee (MTARC) and to reevaluate the cancer classification of lactofen.

The data were presented by Dr. Robert F. Fricke of Reregistration Branch 2 of the Health Effects Division.

II. BACKGROUND INFORMATION

Initial evaluation of the carcinogenic potential of lactofen took place on February 12, 1987 by the Carcinogenicity Peer Review Committee (CPRC). Based on liver tumors seen in both sexes of mice, the CPRC concluded that lactofen should be classified as a B2 (Probable Human Carcinogen) [Attachment 1, Peer Review of Lactofen, Memo from J. Hauswirth to R. Mountfort, dated April 08, 1987, TXR No. : none]. Subsequent quantitative evaluation of the tumor data resulted in a Q_1* of 0.119 $(mg/kg/day)^{-1}$ in human equivalents for lactofen.

The Registrant (Valent U.S.A.) submitted a petition (MRID No. 45160301) requesting that risk assessment for lactofen be based on the MOE approach rather than using a Q_1*. The petition reviewed and summarized earlier data submissions, which supported peroxisome proliferation as the mechanism of action of lactofen.

On January 17, 2001, the MTARC reviewed the merits of the toxicological data supporting peroxisome proliferation as the proposed mode of action for lactofen. Based on the weight-of-evidence from guideline, as well as non-guideline mechanistic studies, the MTARC concluded that peroxisome proliferation is the mode of activation of lactofen [Attachment 2, TXR No. 014590]. The MTARC also recommended that the CARC reevaluate the carcinogenicity classification of lactofen in light of the mode of action of lactofen as it pertains to the formation of liver tumors in mice.

III. STUDIES REVIEWED BY THE CARC

The CARC reviewed summaries of the mechanistic studies as presented in the report of the MTARC . These studies included several non-guideline *in vitro* and *in vivo* mechanism studies that characterized lactofen-induced peroxisome proliferation.

Of the studies evaluated by the CARC, a non-guideline study, in which biochemical and histopathological markers for peroxisome proliferation were measured in both rat and mouse livers (MRID No.: 45283904), was considered most pertinent.

In this study, male and female mice were exposed to technical lactofen at 0, 2, 10, 50, or 250 ppm or to nafenopin (positive control for peroxisome proliferation) at 500 ppm. After 7 weeks of treatment, the mice were sacrificed and the livers examined biochemically and pathologically. Dose-dependent increases were observed (Table 1) in relative liver weights, catalase and acyl CoA oxidase, and carnitine acetyl transferase (females only) was observed. Histological evaluations

(Table 2) also revealed dose-dependent increases in nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining in livers. Nafenopin-treated mice showed significant increases in all of the parameters measured.

These results not only show dose-dependent increases in the parameters measured (see above), but more importantly, a non-linear dose-response curve with a NOAEL. A NOAEL of 2 ppm (0.3 mg/kg/day using a conversion factor of 1 ppm = 0.15 mg/kg/day) was based on increases in relative liver weight (male mice), carnitine acetyl transferase and palmitoyl CoA oxidase (female mice) at a LOAEL of 10 ppm (1.5 mg/kg/day).

The NOAEL for this study was established at 2 ppm (0.3 mg/kg/day), based on increased activities of liver enzymes and increased incidence of liver histopathological findings at the LOAEL of 10 ppm (1.5 mg/kg/day).

IV. CONCLUSIONS:

The CARC concluded that a **NOAEL of 0.3 mg/kg/day should be used to calculate the MOE.** The MOE approach should be used for quantification of risk and the acceptable MOEs will be risk management decisions.

The CARC also concluded that a NOAEL of 0.3 mg/kg/day can be used for chronic and reproductive NOAELs. The NOAEL of 0.3 mg/kg/day is also protective of carcinogenic effects, which has a NOAEL of 10 ppm (1.5 mg/kg/day).

Chronic Toxicological Effects: From the results of a chronic feeding study in the dog, the NOAEL was established at 40 ppm (0.8 mg/kg/day), based on increased incidence of proteinaceous casts in the kidneys and statistically significant decreases in the absolute weight of thyroid and adrenal glands in males at the LOAEL of 200 ppm (3.96 mg/kg/day). The chronic RfD is established at 0.008 mg/kg/day (100x uncertainty factor).

Reproductive Toxicity: Based on the results of a two-generation reproduction study in the rat, the NOAELs for parental, reproductive and offspring toxicity were all established at 50 ppm (2.6 mg/kg/day). Effects observed at the LOAEL of 500 ppm (26 mg/kg/day) included parental toxicity consisting of deaths, decreased body weight/gain and increased incidence of histopathological findings, reproductive toxicity consisting of decreased male fertility, and offspring toxicity consisting of decreased pup body weight, decreased liver weights, and decreased spleen weights.

Carcinogenicity: Based on the results of a carcinogenicity study in the mouse, the carcinogenic effects were seen at doses greater than 10 ppm (1.5 mg/kg/day) and there was an increased incidence of non-neoplastic and neoplastic liver masses seen in both sexes at the LOAEL of 50 ppm (7.5 mg/kg/day).

V. CANCER CLASSIFICATION

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), based on the liver tumors seen in both sexes of the mouse, and in consideration of the mechanistic data provided, the CARC concluded that lactofen should be classified as "**likely to be carcinogenic to humans at high enough doses to cause these biochemical and histopathological changes in the liver of rodents but unlikely to be carcinogenic to humans below those doses causing these changes."** Furthermore, the CARC recommended using an MOE approach for estimating human cancer risk. Therefore, a NOAEL of 2 ppm (0.3 mg/kg/day) should be used for calculating the MOE.

Table 1: Evaluation of Liver Parameters in Male and Female Mice After 7 Weeks of Treatment with Lactofen (Technical, 78.2%; pure, 99.8%) or Nafenopin (NAF)

Sex	Dose (ppm)	Relative Liver Wt (g/100 g)	Catalase	Carnitine Acetyl Transferase	Palmitoyl CoA Oxidase
Male	0	3.9	0.34	2.92	3.1
	2 (Tech)	**4.2**	0.49	**2.96**	5.2
	10 (Tech)	5.0***	0.41	3.87***	6.1
	50 (Tech)	4.5**	0.49	3.27	7.2
	250 (Tech)	7.7***	0.94***	3.1	40***
	250 (pure)	6.2***	0.93***	6.77***	29***
Female	0	4.0	0.22	3.21	2.1
	2 (Tech)	4.1	0.26	3.71	4.3
	10 (Tech)	4.4	0.21	**4.27****	**11*****
	50 (Tech)	4.7**	0.30*	4.68***	13***
	250 (Tech)	6.7***	0.69***	4.53**	35***
	250 (pure)	7.3***	00.44***	7.77***	27***
Male	500 NAF	12.8***	0.70***	8.31***	49***
Female	500 NAF	11.9***	0.76***	9.94***	38***

** $p \leq 0.01$; *** $p \leq 0.001$

[1] Data summarized from Tables 5 and 6 of MRID No. 452383904.

Table 2: Evaluation of Histopathological Parameters in Male and Female Mice After 7 Weeks Treatment with Lactofen (Technical, 78.2%; Pure, 99.8%) or Nafenopin (NAF) [1]

Sex	Dose (ppm)	Nuclear Enlargement	Cytoplasmic Eosinophilia	Hypertrophy	Peroxisomal Staining
Male	0	–	–	–	+
	2 (Tech)	–	–	–	+
	10 (Tech)	±	±	±	+
	50 (Tech)	+	+	+	++
	250 (Tech)	+	++	++	++
	250 (Pure)	++	+++	++	++
Female	0	–	–	–	+
	2 (Tech)	–	–	–	+
	10 (Tech)	±	–	–	+
	50 (Tech)	+	+	+	++
	250 (Tech)	++	++	++	+++
	250 (Pure)	++	+++	+++	++
Male	500 NAF	++	+++	+++	+++
Female	500 NAF	+++	+++	+++	+++

[1] Data summarized from Table 7 and 8 of MRID No. 452383904.

Attachment 1

Peer Review of Lactofen, September 12, 1987

APR 8, 1987

[TXR NO. 0050971]
Subject: Peer Review of Lactofen

From: Judith W. Hauswirth, Ph.D.
Acting Section Head, Section VI
Toxicology Branch/HED (TS-769C)

To: Richard Mountfort
Product Manger #23
Registration Division (TS-767C)

The Toxicology Branch Peer Review Ccumittee met on February 12, 1987 to discuss and evaluate the weight ot the evidence on Lactofen, with particular reference to its oncogenic potential.

. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber

William L. Burnan

Reto Engler

John A. Quest

Esther Rinde

Judith W. Hauswirth

Richard Levy

Louis Kasza

Richard Hill

Robert Beliles

2. Reviewers:(Non-panel members responsible for data presentation,- signatures indicate technical accuracy of panel report).

Joycelyn E. Stewart (Reviewer)

Albin B. Kocialski (Section Head)

Bernice Fisher (Reviewer)

Allen Katz (Reviewer)

3. Peer Review Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Stephen Johnson

Diane Beal

B. Material Reviewed:

The material available tor review consisted of DER's for mouse oncogenicity and lifetime rat feeding studies on Lactofen, summary tables on incidence of neoplastic and non-neoplastic lesions tor both studies, historical control data on pertinent tumors and Toxicology branch "One-Liners".

C. Background Information:

Registration for Cobra . Herbicide (a.i., Lactofen) is being sought by PPG Industries. The herbicide is to be used pre and/or post emergence for control of susceptible broadleaf weeds affecting soybeans, cotton and peanuts. There are no present or previous tolerances in the USA. Lactofen is supplied as a manufacturing use concentrate containing 60% a.i. and as an emulsifiable concentrate containing 2 pounds per gallon a.i.

The chemical name of Lactofen is 1-(carboethyoxy) ethyl 5-(2-chloro-4-(tri-fluoromethyl) phenoxy)-2-nitrobenzoate. Lactofen is also referred to as PPG-844 in this report.

D. Evaluation of Oncogenicity Studies:

Oncogenicity study in mice PPG-844 Technical. Hazleton Laboratories America, Vienna, VA. Study No. 250-152, August 29, 1985. Accession No. 073848.

1. Mouse Oncogenicity Study:

a. Discussion of Study:

Lactofen (78.26% pure) was administered in the diet to groups of 60 male and 60 female Crl:CD-1 (CP,)(SR) mice at levels of 10, 50 and 2-50 ppm for 78 weeks. The study was conducted by Hazleton Laboratories,

America and was dated (3 August 29, 1985. The incidence of hepatocellular-- neoplasm is summarized in the following table.

Incidence of Hepatocellular Neoplasms

	Dose (ppm)			
Tumor Type	0	10	50	250
Males				
hepatocellular				
adenoma	2/50(4)	2/50(4)	5/50 (10)	6/50 (12)
carcinoma	7/50(15)**	7/50(15)	1O/50 (20)	15/50(30)*
total tumor bearing animals	9/50(18)**	9/50(18)	15/50(31)	21/50(42)**
females				
Hepatocellular				
adenoma.	0/5(7(0)	2/50(4)	4/50(8)	3/ 50 (6)
carcinoma	2/50(4)**	1/50(2)	0/50(0)	5/50(10)
total tumor bearing animals	2/50(4)**	3/50(6)	4/50(8)	8/50(16)*

The number in parentheses is the percentage incidence.
Cochran-Armitage Trend test is indicated on Control and Fisher Exact test is indicated on Dose group as: **p<0.01 and * p< O.05

No other tumor response related to Lactofen administration was seen.

Historical control data was provided on liver tumors in CD-1 mice sacrificed after 78 weeks. The data was derived from three studies conducted from 1979-1980 by the performing laboratory.

Historical Control Data
Hepatocellular Tumors

		Total incidence	Range(%)
males	adenoma	3/140	0-4
	carcinoma	19/140	3-17
females	adenoma	3/156	0-7
	carcinoma	5/156	0-14

The incidence of hepatocellular adenomas and carcinomas was well outside the historical control range tor male mice at the highest dose tested (250 ppm). In females, carcinomas and adenomas, considered alone, were within the historical control range.

b. MTD Considerations:

Body weights and food consumption were unaffected by treatment. Survival was slightly affected at the high dose for males and mid and high dose for females but the differences were not statistically significant. Hemoglobin, hematocrit and RBC values were decreased in the high dose females when compared to controls at 58 weeks by 13, 16 and 11%, respectively. This effect was not seen at 78 weeks with the values being only 5, 3 and 2% lower, respectively than corresponding controls. Significantly increased liver/body weight ratios were observed in all treated males an in mid and high dose females.

Non-neoplastic liver pathology in treated groups consisted of the following:

1. Hepatocytomegaly was increased in all treated male groups and in the mid and high dose females;
2. necrosis of individual hepatocytes was increased in the high dose males;
3. Foci/areas of cellular alteration were increased in mid and high dose males and high dose females; and
4. sinusoidal cell pigmentation was increased in all male and female treated groups (Note: the control rate was 22% for males and 42% for females).

In a 90-day CD-1 mouse feeding study, all animals treated with 5000 and 10,000 ppm Lactofen were found dead by week three and week two, respectively. At 2000 ppm statistically significant lower body weights were seen. Also at 20OO ppm an increased incidence of hepatocytic vacuolization, necrosis of individual hepatocytes, hyperplasia of biliary epithelium, hepatocellular swelling and extramedullary hematopoiesis was seen in both sexes.

The Committee concurred that based upon the toxicity, as seen in both the 90-day and oncogenicity studies, that an MTD has not been reached in the latter study and that higher dosages (> 250 ppm) would have been tolerated chronically.

2. Two-Year Rat feeding study:

Two-Year feeding study of the oncogenicity and Chronic Toxicity of PPG-844 in Rats. Hazleton Laboratories, America, Madison, WI. Study N0. 6100-100, July 30, 1995. Accession Nos. 073837-073840.

a. Discussion of Study:

Lactofen (73.5 - 78.3% a.i.) was administered in the diet to Charles River CD Sprague-Dawley derived rats at dosages of O, 50, 500,1000 and 200b.ppm for two years. A total of 84 animals per sex were started in each group. Clinical chemistry, blood chemistry and urinalysis were performed at 6, 12, 18 and 24 months on 8 rats/sex/group. At 6, 12, and 18 months 8

rats/sex/group were sacrificed and liver tissues were examined microscopically.

An increased incidence of "neoplastic" nodules (sometimes referred to as proliferative nodules in the report but listed under neoplastic lesions) was seen in male and female rats at the highest dose tested.

Incidence of Proliferative Nodules (Neoplastic)

	Dosages (ppm)				
	0	50	500	1000	2000
Males	5+/63(8)	1/59(2)	3/60(5)	5/61(8)	15/59(25)**
Females	1/60(2)**	0/59(0)	2/60(3)	2/58(3)	7/60(12)*

+ includes one hepatocellular carcinoma
The number in parentheses indicates the percentage incidence.
Cochran-Armitage Trend test is indicated on Control and Fisher Exact test is indicated on dose group as: **p < 0.01 and *p< 0.05.

Survival analysis of rat data in males indicated a survival disparity. Although the Cochran-Armitage Trend analysis of tumor rates does not adjust for this fact, a more sensitive method of Trend analysis (Peto's) would have resulted in a smaller p value, i.e. a more significant result. This is true because of the earlier occurrence or greater mortality in the highest dose group.

No evidence for early development of neoplastic lesions was apparent from the 6, 12 or 18-month interim sacrifices. The only hepatocellular carcinoma seen was one in the male control group.

Historical control data provided by the performing laboratory in untreated Sprague-Dawley rats from 1980 to 1983 indicated that in males the total incidence of neoplastic nodules from four studies was 7/342 with a range of 1-2% and in females the total incidence of neoplastic nodules was 1/347 and the range was 0-1%.

An increased incidence of endometrial stromal polyps was observed in treated females ($p<0.05$ for all groups by Fisher's exact test) when compared to the controls; however, the incidence was within the historical control range of the performing laboratory and was not considered to be treatment related.

b. MTD Considerations:

The Committee concurred that the high dose in this study approached an MTD. This conclusion was based upon:

- a statistically significant decrease in body weight in both sexes when compared to control values (10-15% in males and 10-18% in females during the study);

- SGOT, SGPT and alanine phosphatase levels were increased in all high dose animals throughout the study; and
- basophilic and eosinophilic foci of cellular alteration were statistically significantly (p<O.05) increased in high close animals of both sexes.

E. Additional Toxicology Information:

1. Metabolism:

Lactofen is rapidly excreted after oral administration with 95% of the radiolabel excreted within 72 hours in rats. Male rats excreted the majority of the radioactivity in feces (56%), while female rats excreted most in the urine (52%).

In mice 65% to 69% of the orally administered radioactivity is excreted within 4 days; male mice excreted 50% and female mice excreted 34% of the absorbed dose in the feces. A small portion remained bound to mouse liver. In male rhesus monkeys fecal and urinary excretions of 14C Lactofen were each approximately 40% of the administered dose and were complete in 144 and 72 hours, respectively after oral administration.

Two types of transformations were observed from the metabolism of PPG844. One route involved hydrolysis of the ethyl ester to form PPG-947. This reaction was followed by further hydrolysis to PPG-84; (Acifluorfen, see #4 below). The second route of metabolism involved reduction of the nitro group or PPG-844 to form PPG-1576, which was further hydrolyzed to PPG-2838 and finally to PPG-2053. The proposed metabolic pathway can be found in Figure 1 (pg. 7).

2. Mutagenicity:

Lactofen did not cause an increase in chromosomal aberrations when tested with and without metabolic activation in CHU cells, was negative both with and without metabolic activation in the CHQ/HGPRT mammalian cell forward mutation assay and did not induce unscheduled DNA synthesis in isolated rat hepatocytes. Lactofen did have a low covalent binding index to mouse liver DNA in vivo and was positive in the Ames Salmonella/microsome plate test to tester strain TA 1538 without metabolic activation but at dosages that precipitated out in the medium (50O0 and 7500 ug/plate).

3. Reproduction and Teratology:

Lactofen was not embryo/fetotoxic when evaluated in rats and rabbits at dosages up to 400 mq/kg and 20 M/kg, respectively. In a 2-generation reproduction study at 500 ppm Lactofen administration resulted in decreased pup weight and increased pup heart and liver weights. The NOEL for these effects was 50 ppm.

METABOLISM OF PPG-844

4. Structure Activity Relationship:

Lactofen and its metabolites are structurally related to four chemicals that are oncogenic in rodents:

- Acifluorfen (the major metabolite of Lactofen; see PPG-647 Fig. 1) induces hepatocellular adenomas and carcinomas in mice but is negative for oncogenicity in rat;
- Nitrofen produces hepatocellular carcinomas in mice and pancreatic carcinomas in rats;
- Oxyfluorfen produces marginally positive liver tumors in mice but is negative in the rat; and
- Fomesafen produces hepatocellular adenomas and carcinomas in mice (Category C oncogen).

5. Special Studies:

Lactofen is not an inducer of microsomal enzyme activity. Lactofen (technical PPG-844, but not pure PPG-844) and Nafenopin, a known peroxisome proliferator, induce catalase activity and increased liver peroxisome and alkaline phosphatase staining in rats and mice. However, liver samples from Chimpanzees treated with Lactofen (technical PPU-844, 5.and 75 mg/kg/day for 90 days) showed no increase in the activity of acyl Coenzyme A oxidase or catalase the increased levels of which are indicative of peroxisome proliferation.

e. Weight of Evidence Considerations:

The Committee, considered the following facts, regarding the toxicology data on Lactofen to be of importance in a weight of the evidence determination of oncogenic potential.

1. Administration of Lactofen in the diet of male and tamale Crl:CD-1(CR)(BR) mice was associated with an increased incidence of hepatocellular tumors (adenomas, carcinomas and total hepatic tumor bearing animals in males and adenomas and total hepatic tumor bearing animals in females).

2. Historical control information from the performing laboratory for the incidence of hepatocellular. tumors in CD-1 mice at 78 weeks indicated that the incidence of hepatocellular adenomas and carcinomas was well outside the historical control range for male mice at the highest dose tested. In mice, carcinomas and adenomas at the highest dose tested were within the historical control range.

3. The MTD was not reached in the mouse oncogenicity study and doses higher than 250 ppm could have been tolerated chronically in CD-1 mice.

4. An increased incidence of "neoplastic" nodules (sometimes referred to as proliferative nodules in the study report but listed under neoplastic lesions) was seen in male and female CD sprague-Dawley derived rats at the highest dose tested (2000 ppm).

5. No evidence tor early development of neoplastic lesions in the liver was apparent from the 6,12 or l8-month interim sacrificers in the chronic rat feeding study.

6. The MTD was approached at the high dose in the rat study based upon increased body weight gain in both sexes (>10%),.increased levels of SGOT, SGPT and alkaline phosphatase in both sexes and increased basophilic and eosinophilic foci of cellular alteration in the livers of both sexes.

7. Lactofen is rapidly excreted in the rat (95% in three days). Excretion in the mouse is slower since only b5-69% is excreted within four days. Some radioactivity remains bound to mouse liver.

8. Lactofen was negative in three short term assays for mutagenicity but was positive at high doses that precipitated in the medium in the Ames Salmonella assay (strain TA 1538) and had, a low covalent binding index to mouse liver DNA in vivo. The latter result corroborates the finding in the mouse metabolism study showing binding radioactivity from 14C-Lactofen in the liver.

9. Lactofen was not embryo/fetotoxic when evaluated in rats and-rabbits. In a 2-generation reproduction study at 500 ppm Lactofen administration resulted in-decreased pup weight and increased pup heart and liver weights. The NOEL for these effects was 50 ppm.

10. Lactofen and its metabolites are structurally related to four chemicals that are oncogenic in rodents. Acifluorfen, Nitrofen, Oxyfluorfen and Fomesafen all induce hepatocellular tumors in mice.

G. Classification of Oncogenic Potential:

The Committee concluded based upon the available evidence that Lactofen meets the criteria of a category B2 oncogen (probable human carcinogen). Lactofen met criteria a for this category in that it induced "an increased incidence of malignant tumors or combined malignant and benign tumors a) in multiple species or strains..." In CD-1 mice an increased incidence of hepatocellular carcinomas in males and an increased incidence of combined hepatocellular adenomas and carcinomas in both males and females was associated with administration of Lactofen. In Charles River CD Sprague-Dawley rats an increased incidence of liver neoplastic nodules was seen in both sexes. Although an increase in malignant tumors was not seen in rats, the Committee felt that a B2 classification was appropriate since a tumor response was seen in two species at the same site. Supportive information was provided by structure activity correlations. Four structurally similar chemicals, Acifluorfen,(the major metabolite of Lactofen), Nitrofen,

Oxyfluorfen and Fomesafen, all produce hepatocellular tumors in rodents. Lactofen was negative in three short term assays for mutagenicity, but did have a low binding index, to mouse liver DNA in vivo. Results of the Ames/Salmonella plate assay were positive in one strain, but only at doses that precipitated in the medium and, therefore, could not be interpreted as a definitive positive result.

Since an MTD was not reached in the mouse oncogenicity study on Lactofen, the Committee felt that the tumor response seen at, the highest could not be considered to be a high dose effect only and that it Lactofen had been tested at higher dosage levels, as well, induction of liver tumors would have been seen at multiple dosage levels.

The Committee discussed the registrant's contention that the mechanism of tumor induction by Lactofen is through peroxisome proliferation and that such a mechanism has a threshold in contrast to a non-threshold mechanism attributed to direct acting carcinogens. The Committee felt that, although this could be part of the mechanism for liver tumor formation by Lactofen, such a mechanism has not been proven to be directly, but only indirectly, associated with liver tumor formation by this and other chemicals[1].

[1] See for example the third edition of Casarett and Doull's Toxicology: The Basic Science of Poisons (edited by C. D. Klaassen, M. 0. Amdur and J. Doull), 3rd edition Macmillan Publishing Company, New York, 1986. p. 136.

Attachment 2

Lactofen - Report of the Mechanism of Toxicity Assessment Committee

January 17, 2001

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 014590

March 12, 2001

MEMORANDUM

SUBJECT: **LACTOFEN**: Report of the Mechanism of Toxicity Assessment Review Committee

FROM: Robert F. Fricke
Reregistration Branch II
Health Effects Division (7509C)

THROUGH: Karl Baetcke,
MTARC, Co-chair,
Health Effects Division (7509C)

and

Pauline Wagner
MTARC, Co-chair,
Health Effects Division (7509C)

TO: Christine Olinger, Risk Assessor
Reregistration Branch I
Health Effects Division (7509C)

EPA Identification Nos
PC Code: 128888
DP Barcode: D267472
Submission: S582396
Case: 819544

Action Requested: The Registrant (Valent U.S.A.) has submitted a petition (MRID No. 45160301) requesting that risk assessment for lactofen be based on the MOE approach rather than using a Q_1* of 0.119 $(mg/kg/day)^{-1}$ in human equivalents (HED Doc No.: 014237, July 12, 2000). The petition reviewed and summarized earlier data submissions, which supported peroxisome proliferation as the mechanism of action of lactofen.

Conclusions: On January 17, 2001, the Mechanism of Toxicity Assessment Review Committee (MTARC) reviewed the merits of the toxicological data supporting peroxisome proliferation as the proposed mode of action for lactofen. Based on the weight-of-evidence from guideline, as well as mechanistic studies ,the MTARC concluded that the evidence presented on peroxisome proliferation indicates that lactofen operates via a mode of action involving PPAR activation.

Evaluation Criteria: Based on the International Life Sciences Institute (ILSI) recommendations), the following criteria were used to assess the mode of action of lactofen:

1.) Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and increased number of peroxisomes as measured by morphometric analysis.

2.) Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

3.) Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially CN-insensitive acyl (palmitoyl) CoA oxidase activities.

Weakness of Submitted Data Supporting Mode of Action on Peroxisome Proliferation:

The only weakness in the submitted data was that BrdU labeling was not performed to evaluate replicative DNA synthesis. The MTARC concluded that this shortcoming did not impact on their conclusions; ample evidence was submitted which demonstrated hepatic hyperplasia after treatment with lactofen.

Strengths of the Submitted Data in Supporting Mode of Action on Peroxisome Proliferation:

1.) The results guideline studies showed that lactofen is neither mutagenic nor genotoxic. A non-guideline study showed equivocal (probably negative) binding of lactofen to DNA.

2.) Changes in liver morphology were observed in both rats and mice treated with lactofen. These effects include: dose-dependent increase in relative liver weights and increased number of peroxisomes as measured by electron microscopic analysis. Further, in mice there was a dose-dependent increase in nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining.

3.) There was evidence of cell proliferation as measured by increased relative liver weights and histological evidence indicating hepatic hyperplasia. These effects were dose-related with a clearly defined threshold.

4.) Dose-dependent increases (particularly in female mice) in the activities of hepatic CN-insensitive palmitoyl CoA oxidase and carnitine acetyl transferase. Dose-dependent increase in CN-insensitive palmitoyl CoA oxidase was also observed in primary rat hepatocytes treated with lactofen.

5.) The doses at which carcinogenicity was observed (mouse, LOAEL = 50 ppm; rat, LOAEL = 2000 ppm) were consistent or higher than the doses which caused peroxisome proliferation.

Other Issues Discussed:

The MTARC recommended that the cancer classification be reviewed by the Cancer Assessment Review Committee. Since the toxicological database for lactofen are relatively extensive, the MTARC further recommended that the Registrant submit the lactofen studies to ILSI as a case study.

Assessment of the Mode of Action for Lactofen

I. Background

A joint meeting (March 29, 2000) of the Mechanism of Toxicity Assessment Review Committee (MTARC) and Cancer Assessment Review Committee (CARC) evaluated the adequacy of the toxicological database for diclofop-methyl in support of peroxisome proliferation as the mechanism of action for liver carcinogenicity. The basis for the evaluation was two detailed literature reviews on the role of peroxisome proliferation in hepatocarcinogenesis. One review [1] by the International Life Sciences Institute (ILSI) evaluated the human cancer risk of peroxisome proliferation, while the other [2] evaluated the human cancer risk of the plasticiser di(2-ethylhexyl)phthalate (DEHP).

As a result of this joint meeting of the MTARC and CARC, criteria (based on the ILSI recommendations) were established which should be met before a non-genotoxic hepatocarcinogenic substance can be classified as a peroxisome proliferator (PP). These criteria are:

1.) Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and increased number of peroxisomes as measured by morphometric analysis.

2.) Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.

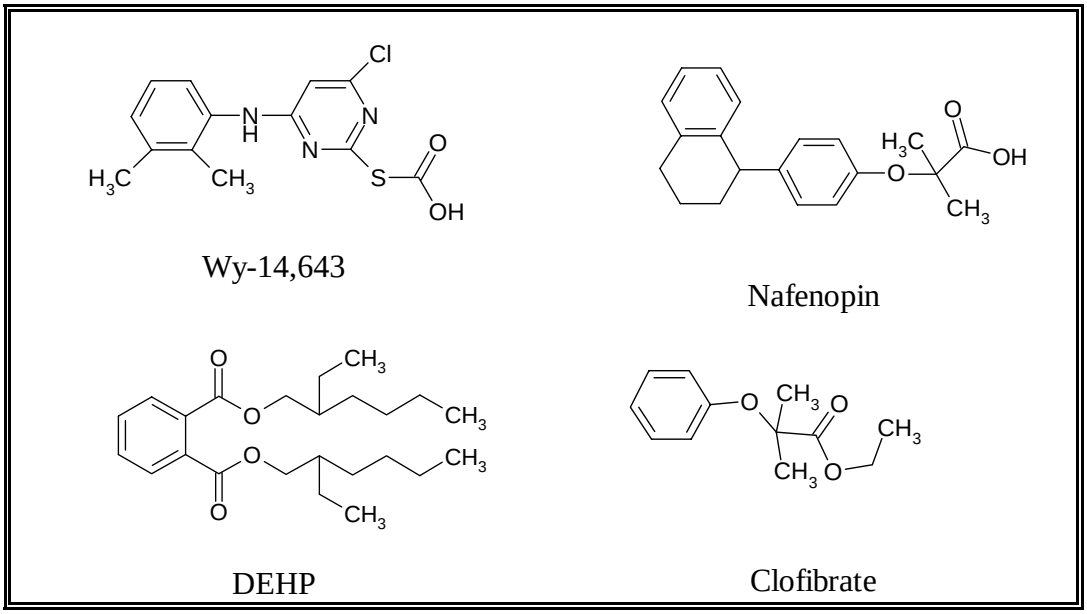
3.) Increased activity of enzymes involved in peroxisomal fatty acid metabolism, especially acyl (palmitoyl) CoA oxidase activities.

II. Mechanism of Action of Peroxisome Proliferators

PPs are a diverse group of chemicals and include synthetic (industrial plasticisers and solvents and hypolipidemic drugs), as well as the naturally occurring compounds (certain fatty acids, prostaglandins, and steroids). Although the synthetic PPs are structurally dissimilar, they share a similarity with fatty acids - a large hydrophobic region and an aliphatic chain with a terminal acidic group, usually carboxylic acid (Figure 1).

Research over the past decade showed that the rodent is highly responsive to PPs and that the liver is the primary target organ. The action of PPs on rodent liver leads to specific and well characterized toxicological events. Short-term effects (as early as one week) on the liver include hepatomegaly due to hypertrophy and hyperplasia, increase in the number and size of peroxisomes, and the transcriptional induction of peroxisomal enzymes (acyl CoA oxidase), endoplasmic reticulum (cytochrome P450) and cytosol (fatty acid binding protein). While these short-term effects on the liver are reversible, long-term exposure leads to the development of hepatic cancer.

Figure 1: Chemical Structures of Some Peroxisome Proliferators



A receptor-based mechanism for the proliferation of peroxisomes was established with the identification of peroxisome proliferator-activated receptor α (PPARα) [3], which is a member of the nuclear receptor superfamily. The binding of PPARα to the retinol X receptor (RXR) produces a heterodimer which becomes fully active with the binding of a PP to the active site. The active PPARα-RXR-PP complex is capable of regulating gene expression through interaction with the peroxisome proliferator responsive elements (PPRE) of target genes.

In a recent review[4], the large number of PPs preferentially activate PPARα; several hypolilidemic drugs (clofibrate) and Wy-14,643 have been shown to directly bind to PPARα. They further point out that the ability of a PP to bind to and/or activate PPARα correlated well with the hepatocarcinogenic potency of the PP. Further definitive evidence for the involvement of PPARα in the development of hepatocellular tumors was demonstrated using transgenic mice which lacked expression of PPARα mRNA.

The introduction of PPARα null mice(-/-) by Lee et al.[5], the obligatory role of PPARα in mediating the cellular effects of PP was established. Short-term exposures to Wy-14,643 or clofibrate showed that while wild-type (+/+) mice developed the classic hepatic effects, the effects in treated null (-/-) mice were comparable to the (-/-) controls. In a chronic toxicity study [6] (+/+) and (-/-) mice were fed either basal diet or diet containing 0.1% Wy-14,643. After one week of treatment, the (+/+) mice had a significant increase (93%) in relative liver weight; further increases in relative liver weights were observed at 5 weeks (245%) and 11 months (355%) of treatment. By contrast, the relative liver weights of treated (-/-) mice were comparable to control (-/-) mice at all timepoints. To evaluate replicative DNA synthesis, the BrdU labeling index was measured in both (+/+) and (-/-) mice. While the BrdU labeling index was significantly increased in (+/+) mice after 1 and 5 weeks of treatment compared to (+/+) controls, the BrdU labeling indexes of treated (-/-) mice were comparable to (-/-) controls at these same timepoints. Gross examination revealed multiple visible nodules in the livers of treated (+/+) mice; microscopic examination showed a 100% incidence of hepatocellular neoplasms. The livers of the treated (-/-) mice were grossly and microscopically comparable to (-/-) control mice.

These data firmly established the central, and necessary, role of PPARα in nongenotoxic hepatocarcinogenesis.

III. Evaluation of Toxicity Database for Lactofen

Both guideline and non-guideline (mechanistic) studies have been reviewed. Pertinent results of guideline studies relevant to the proposed mechanism of action of lactofen are summarized in Table 1.

A. Subchronic Toxicity Studies with Lactofen

1) 4-Week Feeding Study in the Rat (MRID No: 00117563): In a 4-week range-finding study, rats were fed diets at 0, 200,100, 5000 or 10000 ppm lactofen (76% a.i.). Animals in the high-dose group had 100% mortality by day 7. Increased liver and kidney weights were observed at doses of 1000 ppm and higher.

2) Subchronic Feeding Study in the Rat (MRID No: 00117564): In a 90-day subchronic toxicity study, rats were fed diets containing 0, 40, 200 or 1000 ppm lactofen (males: 0, 2.9, 14, 74 mg/kg/day; females: 0, 3.5, 17, or 85 mg/kg/day). At the high-dose level, increased relative (to body weight) liver weights in males (3.4%, control 2.8%) and females (3.1%, control 2.7%) and absolute liver weights in males (18.4 g, control 15.0 g) were observed. Gross findings were observed only in high-dose animals and included dark livers in high-dose males (15/19) and females 4/21); darkened renal cortex was also observed in high-dose males (15/19) and females (3/21). Histopathological evaluation of high-dose animals revealed brown pigmentation in hepatocytes and/or Kupffer cells (males, 17/19; females, 7/21; none in controls), and acidophilic hepatocellular degeneration in males (10/19, none in control) and females (1/21, none in controls) and hyperplasia of bile ducts in males (6/19; controls, 1/20). Other microscopic lesions included brown pigment in tubular epithelium in the kidneys of high-dose animals (males, 12/19; females, 3/21; none in controls).

3) Subchronic Feeding Study in the Mouse (MRID No: 00132882): In a 90-day subchronic toxicity study, mice were fed diets containing 0, 40/2000, 200, 1000, 5000, or 10000 ppm lactofen (calculated doses: 0, 5.7, 29/286, 143, 714 or 1429 mg/kg/day). Further as a result of 100% mortality in 5000 and 10000 ppm males and females, the 40 ppm dose was increased to 2000 ppm at week 5. With the exception of one female, all of the 2000 ppm animals died between 5 and 10 weeks after the increase in the dose. Dose-related increases in serum cholesterol and total protein levels, as well as liver enzymes (ALP, ALT and AST) were observed in 200 and 1000 ppm males and females. Comparable increases in absolute and relative liver weights were seen at 200 ppm (males, 43 - 48%; females, 45 - 53%) and 1000 ppm (males, 198 - 221%; females, 189 - 190%). Gross examination revealed enlarged livers in 200, 1000 and 2000 ppm mice and enlarged spleens in 1000 and 2000 ppm mice. At 2000 and/or 1000 ppm, microscopic examination of the liver showed hepatocytic vacuolization, necrosis of individual hepatocytes, bile retention, coagulative necrosis, hyperplasia of biliary epithelium and increased extramedullary hematopoiesis; the kidney showed nephrosis and cortical fibrosis/scarring.

B. Chronic Toxicity, Carcinogenicity and Reproductive Toxicity Studies with Lactofen

1) Combined 2-Year Chronic Feeding/Carcinogenicity Study in the Rat (MRID No.: 00150329): In this study, rats were fed diets containing 0, 50, 500, 1000, or 2000 ppm (0, 2, 19, 38, and 76 mg/kg/day, based on 20 ppm = 1 mg/kg) lactofen for 104 weeks. Effects seen at 1000 ppm included increased incidence of mottled diffusely dark livers and kidneys, increased aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities, decreased cholesterol, blood urea nitrogen, and total protein globulin levels, and increased incidence in the pigmentation of hepatocytes, Kupffer cells and renal cortical tubule cells. Effects seen at the 2000 ppm were similar to those seen at 1000 ppm, but more severe. Other effects at 2000 ppm included increased incidence in basophilic or eosinophilic foci of cellular alteration and increased incidence of neoplastic liver nodules.

2) 18-Month Carcinogenicity Study in the Mouse (MRID No. 00150343): In this study, mice were fed diets containing lactofen at 0, 10, 50, or 250 ppm (0, 1.4, 7.1, or 36 mg/kg/day, based on 1 ppm = 0.143 mg/kg) for 78 weeks. Effects seen at 50 ppm included increased liver weight, increased incidence of dark colored and/or enlarged livers, hepatocytomegaly (also observed in males at 10 ppm); increased incidences of focal cell alteration (females only), and hepatocellular adenomas ; sinusoidal cell pigmentation in the liver was observed in all dose groups. At the highest-dose tested (250 ppm), the severity of these signs of toxicity was increased. Other effects noted at 250 ppm included increased incidence of non-neoplastic and neoplastic liver masses and increased kidney pigmentation.

3) Chronic Feeding Study in the Dog (MRID No: 41967901): In a one-year toxicity study, dogs were fed diets containing 0, 40, 200, or 1000/3000 (0, 0.79, 4.0, 20/59 mg/kg/day based on 1 mg/kg = 40 ppm) lactofen; because of lack of significant toxicity at 1000 ppm, the dose was increased to 3000 ppm after 4-months of treatment. Dogs fed the 1000/3000 ppm diet showed a slight increase in peroxisomal staining (based on the intensity of brown stippling of D.A.B. stained slides) in the livers. Relative liver weight was increased in high-dose females. There was no evidence of nuclear enlargement, increased mitotic activity, inflammation, or focal necrosis.

Table 1: Summary of Guideline Toxicity Studies with Lactofen

Study (MRID No.)	Liver Weight	Liver Enzymes	Liver Histopathology
4-Week Feeding - Rat (00117563) 0, 200, 1000, 5000, 10000 ppm 100% lethality at 10000 ppm	5000 ppm ♂♂ and ♀♀: Increased relative wt	Not measured	Not measured
13-Week Feeding - Rat (00117564) 0, 40, 200, 1000 ppm ♂♂: 0, 2.9, 14, 74 mg/kg/day ♀♀: 0, 3.5, 17, 85 mg/kg/day	1000 ppm: Increased abs and rel in ♂♂ and rel in ♀♀	1000 ppm ♂♂: increased ALT, AST and ALK	1000 ppm, ♂♂ and ♀♀: Brown pigmentation in hepatocytes and/or Kupfer cells and acidophilic hepatocellular degeneration.
13-Week Feeding - Mice (00132882) 0, 40/2000, 200, 1000, 5000, 10000 ppm 0, 5.7/286, 29, 143, 714 mg/kg/day 40 ppm increased to 2000 ppm at wk 7 100% lethality at HDT	≥200 ppm ♂♂ and ♀♀: Increased relative and absolute wt	≥200 ppm: Increased ALK in ♂♂ and ♀♀, increased ALT and AST in ♂♂ ≥1000 ppm: Increased ALT and AST in ♀♀, increased total cholesterol in ♂♂ and ♀♀	≥200 ppm: Enlarged liver in ♂♂ and ♀♀, extramedullary hematopoiesis in ♀♀, ≥1000 ppm ♂♂ and ♀♀: Hepatocellular vacuolation, bile retention, hepatocellular swelling, and extramedullary hematopoiesis ≥1000 ppm ♂♂: Necrosis of individual hepatocytes,
Carcinogenicity - Mouse (00150343) 0, 10, 50 , 250 ppm 0, 1.4, 7.1, 36 mg/kg/day	≥ 10 ppm ♂♂, ≥50 ppm ♀♀: Increased abs and rel wts	Not measured	Hepatomegaly: ≥10 ppm ♂♂ and ≥50 ppm ♀♀ Focus/Area of Cell Alteration: 50 ppm ♂♂ and 250 ppm ♀♀ Sinusoidal cell pigmentation.: ≥10 ppm in ♂♂ and ♀♀ Necrosis of individual hepatocytes: 250 ppm ♂♂ Hepatocellular Adenoma: ≥50 ppm ♂♂ and ♀♀ Carcinoma: 250 ppm ♂♂ and ♀♀ Combined: 250 ppm ♂♂ and ♀♀

Study (MRID No.)	Liver Weight	Liver Enzymes	Liver Histopathology
2-year Combined Chronic/Onco - Rat (00150329) 0, 50, 500, 1000, 2000 ppm 0, 2, 19, 38, 76 mg/kg/day	≥1000 ppm,♂♂, ≥500 ppm♀♀: Increased relative wt	≥500 ppm,♂♂ and ♀♀: Increased AST, ALT and ALK at 18 mo.	≥1000 ppm,: Pigmentation in hepatocytes(♀♀ only) and Kupfer cells (♂♂ and ♀♀) 2000 ppm: Increased basophilic and eosinophylic (♂♂ only) foci of cellular alteration and proliferative nodules
1-Year Chronic Feeding Study - Dog (41967901) 0, 40, 200, and 1000/3000 ppm 0, 0.8, 4, 20/59 mg/kg/day	1000/3000 ppm ♀♀: Statistically significant increases in the relative liver weight; absolute liver weight not affected.	Not measured	No histopathological findings observed in the liver
2-Generation Reproduction - Rat (00132885) 0, 50 500, 2000 ppm	<u>F1 Adults</u> ≥500 ppm ♀♀ and 2000 ppm ♂♂: Increased relative wt 2000 ppm ♀♀: Increased absolute wt. <u>F1 Pups</u> ≥500 ppm ♂♂: Increased absolute wt 2000 ppm ♀♀: Increased relative wt.	Not measured	<u>F1 Adults</u> 2000 ppm ♂♂ and ♀♀: Centrilobular necrosis/degeneration, brown pigmentation [hemosiderin] in hepatocytes and reticulo-endothelial cells

4) Reproductive Toxicity Study in the Rat (MRID No: 00132885): In this study, rats were fed diets containing lactofen at 0, 50, 500, or 2000 ppm (F0 males/females: 0, 2.6/3.1, 26.2/31.8, 103.5/121.3 mg/kg/day; F1 males/females: 0, 2.7/3.3, 26.732.9, or 115.4/138.9 mg/kg/day). For parental groups at the high-dose level, there was increases in spleen and liver weights; increased incidence of liver [hepatocytic centrilobular degeneration and necrosis] and spleen [extramedullary hematopoiesis] microscopic lesions. For offspring groups no liver toxicity was noted

C. Mutagenicity and Genotoxicity Studies with Lactofen

The guideline and non-guideline studies indicate that lactofen is neither genotoxic nor mutagenic. Equivocal (probably negative) results were observed in an *in vivo* DNA binding assay with radiolabeled lactofen. Results are summarized in Table 2.

Table 2: Summary of Mutagenic and Genotoxic Effects of Lactofen

Assay	Results
Salmonella typhimurium/mammalian microsome mutagenicity assay (MRID No.00150346, 00150346)	Negative: ± S9 including
Salmonella typhimurium/mammalian microsome mutagenicity assay (MRID No. 00150347)	Negative ± S9
In vitro cytogenetic assay with Chinese hamster ovary (CHO) cells (MRID No. 00150348)	Negative for clastogenic effects ± S9
In vitro cytogenetic assay with Chinese hamster ovary (CHO) cells (MRID No. 00150626)	Negative for clastogenic effects ± S9
In vitro unscheduled DNA synthesis in primary mouse hepatocytes (MRID No. 00150349 and 00162141)	Negative
In vitro unscheduled DNA synthesis in primary rat hepatocytes (MRID No. 00150627)	Negative
In vivo DNA covalent binding in mouse liver (MRID No. 00150350)	Equivocal (probably negative) Low level of binding

D. Mechanistic Studies with Lactofen

Several non-guideline *in vitro* and *in vivo* mechanism studies have been submitted which further characterized lactofen-induced peroxisome proliferation. These studies include the measurement of biochemical markers for peroxisome proliferation in the mouse and rat, as well as in primary rat hepatocytes, and a DNA binding study in the mouse. A summary of the results of these mechanistic studies is summarized in the Table 3.

Table 3: Summary of Results for Mechanistic Studies with Lactofen

Study (MRID No)	**Liver Weights**	**Enzyme**	**Pathology/histopathology Electron Microscopy**
Analysis of biochemical and microscopic parameters in Chimpanzee liver (45283901, 45283905) 5 and 75 mg/kg/day	Not measured	Aryl CoA oxidase, catalase and carnitine acetlytransferase activities not affected by treatment	No nuclear enlargement, cytoplasmic eosinophilia or hepertrophy observed in liver biopsies after 0, 1, and 3 months of treatment Slight + response for peroxisomal staining (brown stippling)
Results of the Analysis of Biochemical Parameters in Mouse and Rat Liver Following Exposure to PPG-844 (45283904) Mouse: 0, 2, 10, 50, 250 technical 250 ppm pure Rat: 0 and 2000 ppm technical	Mice at 7-Weeks: ≥10 ppm♂♂ and ≥50 ppm♀♀ Increased rel wt	Catalase and CN-insensitive palmiloyl CoA oxidase increased in♂♂ at 250 ppm and♀♀ at ≥50 ppm.	Pathology: Rats (2000 ppm) and mice (≥50 ppm) showed increased nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomes in number of peroxisomes. EM: Rats (2000 ppm) and mice (250 ppm) showed quantitative increase in the number of peroxisomes
Measurement of Peroxisome Proliferation in Primary Rat Hepatocytes Induced by PPG-844 and Five of its Metabolites (45283902)	Not applicable	Concentration-dependent increase in CN-insensitive palmitoyl CoA oxidase activities with each of the metabolites.	EM: Lactofen (0.01 mM) increased number of peroxisomes and glycogen aggregates. Other metabolites showed occasional peroxisomes

1) Subchronic Toxicity Study in Male Chimpanzees (MRID No.: 45283901, 45283905): In this study, three male chimpanzees per dose group were orally dosed with lactofen at 5 and 75 mg/kg/day for three months, followed by a two-month recovery period. Whole blood was collected before and during treatment for clinical pathological evaluations; liver biopsies were obtained before and after 30 and 90 days of treatment. Compared to pre-treatment values, the activities of acetyl CoA oxidase, catalase and carnitine acetyl transferase in the liver were not affected by treatment. Histopathological evaluation of the liver biopsies did not show any evidence of nuclear enlargement, cytoplasmic eosinophilia, or hypertrophy; peroxisome content in the liver biopsies did not show any change in the pre- and post-treatment evaluations. Further, electron microscopic evaluation of liver did not reveal any evidence of peroxisome proliferation. Based on the results of this study lactofen was not a peroxisome proliferator in chimpanzees.

2) Measurement of Biochemical and Histopathological Markers for Peroxisome Proliferation in Rat and Mouse Livers (MRID No.: 45283904): In a dietary study male and female Crl:CD(S)Br rats and Crl:CD1 mice were fed diets containing lactofen or nafenopin, a positive control for peroxisome proliferation. Biochemical markers of peroxisomal proliferation included measurement of hepatic acyl CoA oxidase, catalase and carnitine acetyl transferase activities. Light and electron microscopic were used to evaluate livers for evidence of peroxisome proliferation

Male and female mice were exposed to technical lactofen at 0, 2, 10, 50, or 250 ppm, pure lactofen at 250 ppm, or nafenopin, at 500 ppm. After 7 weeks of treatment, male and female mice showed significant biochemical and pathological effects on the liver. Dose-dependent increases in relative liver weights, catalase and acyl CoA oxidase were observed in males and females; females also showed a significant increase in carnitine acetyl transferase (Table 4). Liver histology (Table 5) revealed significant, dose-dependent increases in nuclear enlargement, cytoplasmic eosinphilia, hypertrophy and peroxisomal staining. Nafenopin-treated mice showed significant increases in all of the parameters measured.

Similar findings were also observed in an 8-week study, in which rats were fed diets containing lactofen at 0 or 2000 ppm or nafenopin at 500 ppm. Lactofen-treated rats had significantly increased relative liver weights, carnitine acetyl transferase and acyl CoA oxidase (Table 6). Histological examination revealed increased incidence of nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining (Table 7); catalase activity was not affected by treatment. Nafenopin-treated rats showed significant increases in all of the parameters indicative of peroxisome proliferation

Electron microscopic analysis was performed on the livers of male rats and mice. As a quantitative measurement of peroxisome proliferation, the ratio of peroxisomes to mitochondria were determined. The ratios were 1/4.7 (21%) and 1/3.0 (33%) for control and treated (2000 ppm, 8 weeks) male rats, respectively, and 1/4.8 (21%) and 1/1.5 (66%) for control and treated (250 ppm, 7 weeks) mice, respectively.

Table 4: Evaluation of Liver Parameters in Male and Female Mice After 7 Weeks of Treatment with Lactofen (Technical, 78.2%; pure, 99.8%) or Nafenopin (NAF)

Sex	Dose (ppm)	Relative Liver Wt (g/100 g)	Catalase	Carnitine Acetyl Transferase	Palmitoyl CoA Oxidase
Male	0	3.9	0.34	2.92	3.1
	2 (Tech)	4.2	0.49	2.96	5.2
	10 (Tech)	5.0***	0.41	3.87***	6.1
	50 (Tech)	4.5**	0.49	3.27	7.2
	250 (Tech)	7.7***	0.94***	3.1	40***
	250 (pure)	6.2***	0.93***	6.77***	29***
Female	0	4.0	0.22	3.21	2.1
	2 (Tech)	4.1	0.26	3.71	4.3
	10 (Tech)	4.4	0.21	4.27**	11***
	50 (Tech)	4.7**	0.30*	4.68***	13***
	250 (Tech)	6.7***	0.69***	4.53**	35***
	250 (pure)	7.3***	00.44***	7.77***	27***
Male	500 NAF	12.8***	0.70***	8.31***	49***
Female	500 NAF	11.9***	0.76***	9.94***	38***

** $p \leq 0.01$; *** $p \leq 0.001$

[1] Data summarized from Tables 5 and 6 of MRID No. 452383904.

Table 5: Evaluation of Histopathological Parameters in Male and Female Mice After 7 Weeks Treatment with Lactofen (Technical, 78.2%; Pure, 99.8%) or Nafenopin (NAF) [1]

Sex	Dose (ppm)	Nuclear Enlargement	Cytoplasmic Eosinphilia	Hypertrophy	Peroxisomal Staining
Male	0	–	–	–	+
	2 (Tech)	–	–	–	+
	10 (Tech)	±	±	±	+
	50 (Tech)	+	+	+	++
	250 (Tech)	+	++	++	++
	250 (Pure)	++	+++	++	++
Female	0	–	–	–	+
	2 (Tech)	–	–	–	+
	10 (Tech)	±	–	–	+
	50 (Tech)	+	+	+	++
	250 (Tech)	++	++	++	+++
	250 (Pure)	++	+++	+++	++
Male	500 NAF	++	+++	+++	+++
Female	500 NAF	+++	+++	+++	+++

[1] Data summarized from Table 7 and 8 of MRID No. 452383904.

Table 6: Evaluation of Liver Parameters in Male and Female Rats After 8 Weeks of Dietary Treatment with Lactofen (Technical, 78.2%) or Nafenopin (NAF)

Sex	Dose (ppm)	Relative Liver Wt (g/100 g)	Catalase	Carnitine Acetyl Transferase	Palmitoyl CoA Oxidase
Male	0	3.0	0.37	3.5	3.4
	2000	4.3***	0.36	6.4***	8.6**
Female	0	2.8	0.20	7.3	5.3
	2000	3.8***	0.19	2.4***	18.3***
Male	500 NAF	5.4***	0.60***	2.9	13.3***
Female	500 NAF	3.7***	0.30***	10.2***	12.5**

** $p \leq 0.01$; *** $p \leq 0.001$

[1] Data summarized from Table 11 of MRID No. 452383904.

Table7: Evaluation of Histopathological Parameters in Male and Female Rats After 8 Weeks Treatment with Lactofen (Technical, 78.2%) or Nafenopin (NAF) [1]

Sex	Dose (ppm)	Nuclear Enlargement	Cytoplasmic Eosinphilia	Hypertrophy	Peroxisomal Staining
Male/	0	–	–	–	+
	2000	++	++	++	++
Female	0	–	–	–	+
	2000	++	++	++	++
Male	500 NAF	++	++	++	+++
Female	500 NAF	++	++	++	++

[1] Data summarized from Table 12 of MRID No. 452383904.

3.) Measurement of Biochemical Markers for Peroxisome Proliferation in Primary Rat Hepatocytes (MRID No.: 45283902): In this study, primary cultures of rat hepatocytes were exposed to PPG-844 (lactofen, 99.8%) or five of its principal animal metabolites (structures Figure 2): PPG-947 (desethyl lactofen 98.9% purity), PPG-847 (acrifluorfen, 100% purity), PPG-1576 (amino lactofen, 95.6% purity), and PPG-2053 (amino acifluorfen, 94.6% purity), PPG-2838 (desethyl amino lactofen, 70.6% purity). Because of the low purity of PPG 2838, it was excluded from the definitive study. CN-insensitive palmitoyl CoA oxidase activity, a biomarker for peroxisome proliferation was measured after 68 hr of exposure. Solvent (DMSO) and positive (clofibrate) were also evaluated.

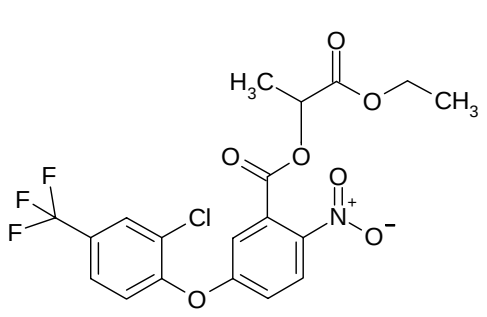
Additionally, control, lactofen, metabolite and clofibrate-treated hepatocyte were examined using an electron microscope.

CN-insensitive palmitoyl CoA oxidase data are summarized in Table 8. Of the test chemicals evaluated, treatment with lactofen resulted in greater than 5-fold, concentration-dependent increase in palmitoyl CoA oxidase activity compared to the solvent control. the highest increase for the metabolites was approximately 3-fold. Decending order of potency, based on palmitoyl CoA oxidase activity, was PPG-844, -947, -847, -1576 and -2053.

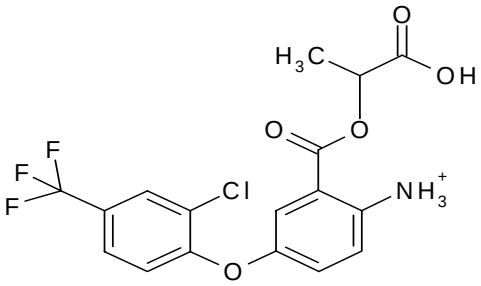
Clofibrate produced significant increases (6.9 to 7.7-fold) in enzyme activity at both of the concentrations test.

Electron micrographs of lactofen and clofibrate-treated hepatocytes revealed an increased numbers of dense, anucleoide peroxisomes compared to the solvent control. Additionally, lactofen-treated hepatocytes showed increased amounts of cytoplasmic glycogen aggregates. Treatment with the metabolites did not cause any increase in the number of peroxisomes relative to the control.

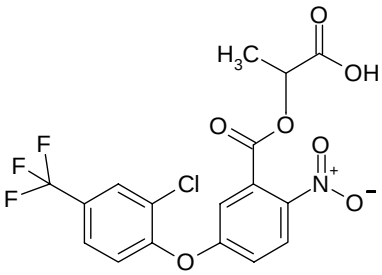
Figure 2: Structures of Lactofen and Primary Metabolites



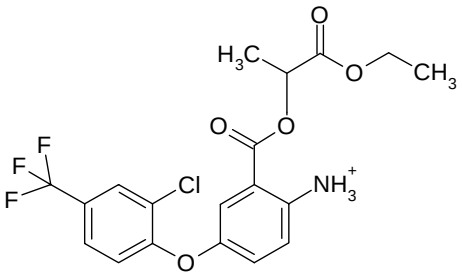
Lactofen
(PPG-844)



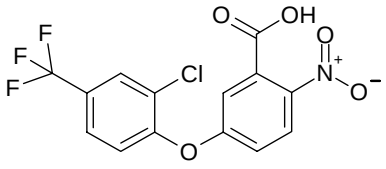
Desethyl amino lactofen
(PPG-2838)



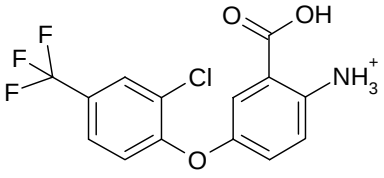
Desethyl Lactofen
(PPG-947)



Amino Lactofen
(PPG-2838)



Acifluorfen
(PPG-847)



Amino Acifluorfen
(PPG-2053)

Table 8: Effect of Lactofen and Metabolites on CN-Insensitive Palmitoyl CoA Oxidase Activity (nmol/min/mg protein) in Primary Rat Hepatocytes

Addition	Concentration, mM			
	0.003	**0.01**	**0.03**	**0.10**
PPG-844 (Lactofen)	1.55*	2.47*	NT	2.63*
PPG-947	1.61*	1.68*	NT	1.74*
PPG-847	NT [2]	1.35*	1.35*	1.75*
PPG-1576	NT	1.26*	0.74[3]	1.57[3]
PPG-2053	NT	0.86	0.98[3]	1.46*
Controls				
Solvent Control (DMSO)		0.50		
Positive Control Clofibrate, 0.16 mM		3.46*		
Clofibrate, 0.50 mM		3.86*		

[1] Data summarized from Table 2 of MRID No. 45283902.
[2] NT = not tested
[3] Mean of two observations, significance not determined.
* Significantly different from solvent control, $p \leq 0.05$.

IV. Other Modes of Action

Studies with transgenic mouse confirmed that essentially all of the of the effects of PPs in rodent liver are mediated by PPARα.

V. Recommendations to the MTARC

Based on the weight-of-the-evidence of the toxicity database, there are sufficient data to classify lactofen as a non-genotoxic hepatocarcinogen in rodents with peroxisome proliferation being a plausible mode of action.

VI. Relevancy to Humans

The human relevancy of the role of peroxisome proliferators was not addressed, and will be deferred pending a proposed ILSI evaluation of pertinent data.

VI. References

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